Research Report

Ovum pick up, in vitro embryo production, and embryo transfer as value-added theriogenology service in miniature Hereford cattle operation

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Abstract

Goal was to provide evidence for adopting assisted reproductive technologies (ART), ovum pick up (OPU), in vitro embryo production (IVP), and embryo transfer (ET) in small beef cattle operations as value-added service. Day 7 embryos (n = 14) were produced via IVP utilizing oocytes (n = 87) collected by OPU from miniature Hereford donor cows (n = 3; mean age: 7 years) and fertilized with semen from miniature Hereford bulls. Irish Black (n = 8; 14 months) and Hereford (n = 1; 20 months) recipient heifers were synchronized using 5-day Select-Synch + controlled internal drug release (CIDR) protocol. On day 9 after CIDR removal, heifers (n = 7) with a corpus luteum (> 2 cm in size) received an embryo in the ipsilateral uterine horn. Pregnancy/ET was 57.1% (4/7). Economic advantage (increase in number of offspring) was realized comparing OPU-IVP and artificial insemination procedures. These ART technologies (OPU, IVP, and ET) can be utilized in genetically superior cows as value-added theriogenology service to increase their offspring.

Keywords: Miniature Hereford, synchronization, ovum pick-up, in vitro, embryo transfer

Introduction

Transvaginal ultrasound-guided follicular aspiration and oocyte retrieval are performed for ovum pick up (OPU) and in vitro embryo production (IVP). Ovum pick up technique was first described in humans in 1940s[1] and was performed in cattle by the 1980s.[1] Collected oocytes are matured, fertilized, and cultured in vitro up to transferable embryo stage. Follicle stimulating hormone (FSH) is commonly used to improve oocyte quality,[2] by synchronizing follicular wave emergence and maximizing the number of competent oocytes. Follicular stimulation and oocyte collection methods vary based on cattle breed, collection frequency, and veterinarian preference. There are several methods primarily used (e.g. a slow-release porcine-derived FSH formulation given as a single injection or traditional twice-daily FSH treatment regimen given over 4 days[3]). However, OPU can also be performed at various stages of the estrous cycle without hormone use.[4]

Use of OPU-IVP technology has increased greatly in the last decade due to improved methods and ability to implement the procedure in the field. In recent years, over 1 x 10^6 embryos[5] were produced per year via OPU and IVP. Number of IVP embryos became equal to or greater than those produced in vivo in 2016.[5-7] Globally, the total number of embryos produced has increased each year in the last decade.[5-7] Lately, more IVP embryos are produced compared to in vivo embryos as there are more preference and demand for IVP embryos in the field.[5-7] In USA alone, 133,000 IVP and 301,000 in vivo embryos were produced in 2020.[8] A huge advantage of OPU-IVP is that numerous embryos can be produced from animals of various reproductive statuses, including pregnant cows up to 100 days in pregnancy, postpartum cows, and prepubertal heifers,[9] making this technology a viable option in smaller cattle operations. Several calves can be produced frequently from valuable animals, giving an economic advantage to the farm. Our objective was to demonstrate the application of OPU-IVF in the field as value added theriogenology service.

Materials and methods

Since data collected from routine assisted reproduction procedures from a private farm for commercial purposes were used,

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institutional animal care and use committee approval is not required, and the study is exempt.

Donor animals and OPU-IVF method

Three donor animals, from a small, registered miniature Hereford cattle herd (herd size; n = 40), were selected based on phenotype, genetic value, and a history of successfully delivering offspring. Selected donors were multiparous miniature Hereford cows (4, 7, and 10 years of age; 2 polled and 1 horned). Each animal was subjected to an OPU procedure after receiving 120 mg of intramuscular FSH [3.5 ml; 350 mg NIH-FSH-P1 diluted in 10 ml of slow-release formula (20 mg/ml hyaluronan); Vetoquinol, TX, USA] 3 days prior to the day of follicle aspiration.[10,11] Before the procedure, epidural anesthesia was induced by injecting 5 ml of 2% lidocaine (Vet One, Boise, ID, USA) into the sacrococcygeal epidural space. Follicles > 2 mm were identified via transvaginal ultrasonography (SonoScape S8, Universal Imaging Inc., Bedford Hills, NY, USA) with convex transducer probe holder (Minitube USA Inc., Verona, WI, USA) after ovary was aligned against the transducer via transrectal manipulation. A long-beveled needle (18 G x 3″ for bovine OPU, Minitube USA Inc.) was used to puncture the vaginal wall at the anterior vaginal fornix and outer layers of the ovarian follicle. Cumulus oophorus complexes were aspirated by an aspiration pump (Cook® Vacuum pump, Cook Medical LLC, Bloomington, IN, USA) for OPU after creating an optimal negative pressure (60 ± 5 mm Hg), one end of a tube was connected to an aspiration needle and the other end to the vacuum pump, to facilitate collection of oocytes into a 50 ml collection tube containing transport medium (BO-HEPES-IVM, Agtech Inc., Manhattan, KS, USA). Collected oocytes were graded and viable oocytes were transported to a private commercial IVF laboratory in a portable incubator. Harvested oocytes were washed, graded, and allowed to mature in the maturation medium (BO-IVM, Agtech Inc.). Sperm from conventional semen or reverse sex-sorted semen from a miniature Herford bull were used for fertilizing oocytes (day 0); oocytes were vortexed to remove their cumulus layers. Conventional semen was used for fertilization of oocytes from 2 polled animals, whereas reverse x-sorted semen (X-RSS) was used for fertilization of oocytes from the horned animal. After in vitro culture and daily assessment for embryo development, embryos were staged and graded[12] on day 7. Grades 1 and 2 embryos at developmental stages 5, 6, and 7 were selected and cryopreserved in ethylene glycol (EG) for direct transfer. Briefly, embryos were placed into a controlled rate freezer at a temperature of –6 °C. Ice crystal formation was induced in EG surrounding embryo. Crystallization increased EG concentration outside the embryo resulting in further cellular dehydration. Embryos were cooled at a rate of 0.5 °C/minute, enabling further dehydration, to a temperature of –34 °C before plunging into liquid nitrogen (–196 °C).

Recipient animals and embryo transfer procedure

Nine 14-month virgin Irish Black heifers and 1 20-month Hereford heifer were enrolled as embryo recipients. Heifers received primary and booster vaccinations against reproductive and respiratory pathogens (bovine rhinotracheitis, bovine virus diarrhea virus types 1 and 2, infectious bovine rhinotracheitis virus, parainfluenza 3 virus, bovine respiratory syncytial virus, *Leptospira canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae,* and *L. pomona* [Bovi-Shield GOLD® FP® 5 VL5, Zoetis]), 13 and 9 weeks prior to embryo transfer, respectively. They were concurrently

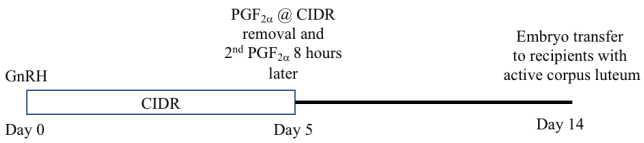

Figure. Select-Synch + CIDR protocol.

vaccinated against clostridial species (*chauvoei*, *septicum*, *haemolyticum*, *novyi*, *sordellii*, *perfringens* type C and D, and *tetani* [Ultrabac® 8, Zoetis]). Crystal-Phos® 8 mineral (Cyrstalyx, Mankato, MN, USA) was available freely and heifers were maintained on pasture. Reproductive tract score (RTS),[13] body condition score (BCS),[14] pelvic area,[15,16] and temperament score[17] were assigned to recipients as part of farm heifer selection protocol. One Irish Black heifer was excluded due to advanced pregnancy at RTS.

Estrus was synchronized in recipient heifers using the 5-day controlled internal drug release (CIDR) Select-Synch[18] protocol (Figure). Briefly, heifers received a CIDR insert (EAZI-BREED CIDR®, 1.38 g of progesterone, intravaginal; Zoetis Animal Health, Parsippany, NY, USA) and 100 µg gonadorelin (intramuscular GnRH, Factrel®, 2 ml; Zoetis Animal Health) on day 0. On day 5, the CIDR device was removed, and 500 µg cloprostenol (intramuscular $PGF_{2\alpha}$, Estrumate®; 2 ml, Merck Animal Health) was given. Eight hours after CIDR removal and initial $PGF_{2\alpha}$ treatment, a second dose of $PGF_{2\alpha}$ was given. It was predicted that heifers would exhibit estrous behavior near day 7 and ovulate ~ 30 hours after estrus onset.[19]

Nine days after CIDR removal, heifers were examined via transrectal palpation and transrectal ultrasonography (SonoScape S8) for corpus luteum. Seven of 9 heifers had an active corpus luteum, deemed as functional based on a size > 2 cm. Heifers were given 2% lidocaine (4 - 6 ml; Vet One) epidurally between caudal vertebrae 1 and 2. Each embryo was identified from the straw label with dam and sire information, stage, grade, and collection date. Embryos selected were thawed in a water bath at 36°C for 30 seconds and were transferred as far cranial as possible into the horn ipsilateral to the identified corpus luteum. Recipients age, embryo stage, and grade are listed (Table 1).

After embryo transfer, heifers were allowed back onto pasture with no further manipulation until pregnancy determination. Pregnancy was determined by measuring pregnancy-specific protein B[20] (BioPRYN®, BioTracking, Moscow, ID, USA) 3 weeks after embryo transfer. Pregnancy was confirmed again at 5 months of pregnancy via transrectal ultrasonography.

Economic analysis

Economic advantage was determined using a crude economic analysis with following assumptions.

OPU-IVP procedure

1. One OPU procedure + drug @ $225
2. Four transferable embryos
3. Freezing + embryo transfer cost @ $170/embryo
4. Four recipients (15 months) purchased @ $1,200/recipient
5. ET success at 50%, 4 embryo transfer result in two calves
6. Newborn embryo calf market price @ 5,100/calf

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7. $800 to cover expenses for a donor cow from 15 months - 2 years (breeding to calving).
8. Sale of 1 open cow at $1,200

Artificial insemination procedure

1. $1,800 to cover expenses for 1 heifer from calf to 2 years (birth to calving)
2. Estrus synchronization and AI success at 50%, 2 AIs result in 1 calf
3. Newborn AI calf market price @ 3,000/calf
4. Sale of 1 open cow at $1,200

Recipients purchase cost ($1,200), expenses from calf to 2 years ($1,800), and from breeding (15 months) to 2 years ($800) were determined considering feed cost, pasture cost, yardage, vaccine/drugs; veterinary expense, death loss, trucking, and interest.

Results

From 3 donor animals, 87 COCs were harvested via OPU for oocytes, and 14 transferrable embryos were produced from the IVP procedure (Table 2). Forty-seven oocytes (fertilized with conventional semen) from 2 polled donor cows resulted in 11 transferrable embryos, whereas 40 oocytes (fertilized with X-RSS) from the horned donor cow resulted in 3 transferrable embryos. There was a trend toward a significance (Fisher's exact; p = 0.07) in blastocyst rate between conventional semen versus X-RSS (Table 3).

Four of the 7 recipient heifers were pregnant (57.1%). Confirmation via transrectal ultrasonography was performed at 5 months of pregnancy (pregnant heifers had fetuses and placentomes of appropriate size).

An association between BCS and RTS to conception was observed. Heifers with moderate to good body condition were more likely to have a CL at embryo transfer. Heifers with an RTS of 5 had higher heifer pregnancy rates/embryo transfer than their counterparts.

Economic analysis based on the assumption for OPU-IVF and artificial insemination procedures is provided (Table 4a and 4b). Approximate economic advantage was estimated based on the costs of average embryo and calf value reported by the farm.

Economic advantage (revenue OPU-IVF – revenue AI) = **$ 3,469.00 – $ 432.00 = $ 3,037.00**

Table 1. Recipients age, semen type used for in vitro fertilization (IVF), embryo stage, and grade

Heifer ID	Age (months)	Semen type used for IVF	Embryo stage	Embryo grade
1	14	CS	5	1
2	14	CS	6	1
3	14	CS	7	2
5	14	CS	7	1
6	14	CS	6	1
8	14	CS	5	1
11	20	X-RSS	6	2

CS: Conventional semen; X-RSS: reverse x-sorted sexed semen

Table 2. Number of oocytes harvested and total and transferable embryos from 3 donor cows

Heifers	Oocytes recovered	Embryos		Blastocyst rate
		Transferable	Numbers (stage, grade)	
1	24	4	2 (6, 1) and 2 (5, 1)	16.7% (4/24)
2	23	7	2 (7, 1), 1 (7, 2), and 4 (6, 1)	30.4% (7/23)
3	40	3	1 (6, 2) and 1 (5, 2)	7.5% (3/40)

Table 3. Embryo development from conventional semen and reverse x-sorted sexed semen

Semen type	Oocytes	Transferable embryos	Blastocyst rate*
CS	47	11	23.4% (11/47)
X-RSS	40	3	7.5% (3/40)

CS: conventional semen; RSS: reverse x-sorted sexed semen
*CS versus RSS (p = 0.07)

Table 4a. Economic analysis for profit estimation generated after implementing OPU-IVP procedures (expenses and revenues considered, based on the assumptions listed*)

Items	OPU-IVF	Total
Expenses		
Donor		
OPU procedure + drug cost	225.00	
Freezing + embryo transfer $170 × 4	680.00	
Shipping	40.00	
Labor ($15/hour × 1 hour)	15.00	
Syringe and needle	2.50	
Recipient		
Purchase cost ($1,200/head × 4)	4,800.00	
Expenses (15 month - 2 year) $800 × 4	3,200.00	
Drug cost		
CIDR ($14.50 × 4)	58.00	
GnRH ($2.50 × 4)	10.00	
$PGF_{2\alpha}$ × 2 ($5.50 × 4)	22.00	
Lidocaine (0.50 × 4)	2.00	
Blood test (BioPRYN) ($5 × 4)	20.00	
Ultrasonography ($6 × 4)	24.00	
Labor ($15/hour × 2 hours)	30.00	
Syringe and needle	2.50	
Total expenses		**9,131.00**
Income		
Calf sale value ($5,100 × 2)	10,200.00	
Open cow sale ($1,200 × 2)	2,400.00	
Total income		**12,600.00**
Profit		**3,469.00**

*Refer materials and methods for the list of assumptions

Discussion

Advanced reproductive technologies can accelerate genetic gain by increasing the number of offspring within a shorter period from genetically superior dams.[21] In this report, donor recipients were selected for desired phenotype and genetic importance within the herd, and OPU-IVF technologies were utilized to maximize their reproductive efficiency.

Pharmaceutical manipulation of estrus or ovulation, AI application, and use of gender-selected semen are well-established methods for improving genetic potential in beef cattle herds;[22] however, these technologies can only produce 1 calf a year per cow using a conventional approach. Success of OPU-IVF technologies, as noted in this report, provide an additional avenue for producers to potentiate the genetics of their most valuable animals.

Cows, similar to those included in this report, are limited to produce ~ 7 high value, show calves with AI technology during their lifetime; however, implementation of OPU-IVP technologies significantly increased the number of offspring[21] and consequently herd profit. Three of the pregnancies in this study were from a single oocyte collection procedure from a cow with great genetic importance. This offered a monetary advantage to the operation by a three-fold increase in calves produced from this particular animal in a year. The 4th embryo pregnancy was achieved from an older cow that was retired from breeding due to predicted poor ability to produce good quality day 7 embryos or to carry pregnancy; however, use of OPU-IVF technologies effectively lengthened cow's reproductive life.

To estimate the monetary benefit, we performed a crude economic analysis comparing OPU-IVP and AI procedures. The OPU-IVF procedures resulted in ~ $3,000 more profit than the AI procedure. Readers should exert caution while interpreting economic analysis since economic benefit would vary due to year-to-year differences in costs and calf market value.

In general, the breakeven cost for raising a replacement heifer can be met after production of 1.5 - 2 offspring by that heifer. Therefore, the profit can be realized when a 3-year cow has a successful weaned second calf. It should be noted that the profit depends on the sale price of a calf; several factors including age of calf at sale, season of sale, genetic composition of calf, gender and sale options such as sale for slaughter, sale as

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Table 4b. Economic analysis for the estimation of profit generated using artificial insemination (expenses and revenues considered based on the assumptions listed*)

Items	AI	Total
Expenses		
Expenses (from birth to 2 years) × 2	3,600.00	
Drug cost		
CIDR × 2 ($14.50 × 1)	29.00	
GnRH × 2 ($2.50 × 2)	10.00	
$PGF_{2\alpha}$ × 2 ($5.50 × 2)	11.00	
Semen ($20/straw) × 2	40.00	
AI fee ($7/head) × 2	14.00	
Syringe/needle	10.00	
Labor ($15/hour × 2 hours)	30.00	
Ultrasound ($6/head) × 2	24.00	
Total expenses		**3,768.00**
Income		
Calf sale value ($3,000 × 1)	3,000.00	
Open cow sale ($1,200 × 1)	1,200.00	
Total income		**4,200.00**
Profit		**432.00**

*Refer materials and methods for the list of assumptions

breeding bull or replacement heifer determines the sale price. The biggest loss to an operation is culling a 3-year open cow due to its reproductive failure. In this report, 3 calves were produced from 1 OPU-IVF procedure from 1 animal, the breakeven cost was achieved much earlier.

Assessment of body condition score and reproductive status of recipients by evaluating the reproductive tract score can be valuable in recipient selection. In this report, moderate to good body condition and reproductively mature heifers were highly reflective of conception success.

In general, a lower blastocyst rate with sex-sorted semen (2 x 10^6 sex-sorted sperm/straw) compared to CS has been reported.[23] However, the pregnancy and efficiency were comparable between CS and sex-sorted sperm (SexedULTRA™ with 4 million sex-sorted sperm/straw).[23] In the current study, reduced fertilization and embryo production were realized ($p < 0.05$) for RSS compared to CS. Although oocytes recovered were graded and selected for further procedures, variation in donor animals plausibly contributed to this difference in the outcome.[24] Reduced blastocyst rate for RSS compared to CS, in 3 different bulls, 15 versus 35%, 34 versus 50%, and 34 versus 41%, respectively, whereas no differences were observed between RSS and CS for pregnancy rate.[25] Decreased motility, viability, and speed of sperm were reported[26] when RSS were subjected to an additional freezing procedure. The potential of refreezing RSS without losing sperm fertilization capability might overcome this limitation.

Conclusion

Reproductive technologies (OPU-IVF) can be successfully utilized as a value-added theriogenology service to produce genetically superior offspring in smaller beef operations. Use of advanced reproductive technologies in genetically superior cows can increase the herd value and profit via the production of an increased number of valuable offspring.

Conflict of interest

None to report.

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